Hepatic gene expression changes associated with in utero arsenic exposure accelerated atherosclerosis in the ApoE-knockout mouse

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Outline

- Background
- Model for transplacental arsenic induced atherogenesis
- Preliminary genomics data on altered liver gene expression
Arsenic Is Widely Distributed & Causes Disease

- >140 million people exposed worldwide
- Variable disease outcome

⇒ Known human carcinogen
⇒ Known human atherogen
⇒ Probable human teratogen

http://phys4.harvard.edu/~wilson/arsenic/arsenic_project_introduction.html
Atherosclerosis Causes Arterial Wall Thickening, Lumen Occlusion and Diminished Blood Flow

Consequences of atherosclerosis:
- Myocardial Infarction
- Stroke
- Kidney failure
- Peripheral artery disease

http://www.h4heart.com/h4heart/articleDisplay.aspx?articleSubDetailID=CC_TPS_Pha_6
http://www.healthcentral.com/high-blood-pressure/index-000102_1-145.html
http://inventorspot.com/articles/scientists_work_stroke_vaccine_37775

Arteries become narrowed and blood flow decreases in atherosclerosis.
Atherosclerosis Is Initiated by Fat Deposition and Macrophage Invasion of Intima

http://www.lipo-search.com/eng/lipo.html
Arsenic-Induced Peripheral Vascular Disease

Blackfoot disease in endemic area of Taiwan

Gangrene examples from Bangladesh

http://www.physics.harvard.edu/~wilson/arsenic/Arsenic%20Foundation.html
Arsenic and Vascular Disease

- **Taiwan**: Dose-response relationships between arsenic exposure and both peripheral vascular disease (Tseng, 1996) and ischemic heart disease mortality (Chen, 1996)

- **Michigan**: Elevated mortality rates for both males and females for all diseases of the circulatory system (Meliker, 2007)

- **Spain**: Elevated cardiovascular mortality rates for those exposed to >1 μg/L (Navas-Acien, 2009)
Advanced Atherosclerosis and Myocardial Infarction in Infants

- **Chile**: Myocardial infarction in infants whose mothers consumed water with high levels of arsenic (Rosenberg, 1973, 1974)

Hypothesis

Fetal arsenic exposure accelerates and exacerbates atherogenesis
ApoE Knockout Mouse

- Developed in mid-'90’s in Breslow’s lab
  - Wild type mice are atherosclerosis resistant
- Hyperlipidemic without high fat diet
- Spontaneous atherosclerosis by 24 weeks
- Fatty streaks start ~12 weeks
- High fat diet accelerates atherogenesis
Experimental Design

• 2 exposure groups:
  – *In utero* arsenic exposed (49 ppm As in tap water *ad libitum* to pregnant dams)
  – Control (tap water)
• Mice maintained on normal chow
• Mice sacrificed before ApoE^-/- normally show disease (acceleration, age 10 wks) and after disease is normally evident (exacerbation, age 16 wks)
Arsenic Exposure Protocol

NaAsO₂: 85 mg/L

At 10 weeks of age, control ApoE(-/-) mice show no gross signs of disease; 85 mg/L NaAsO₂ = 49 ppm As
Liver Arsenic Levels

Mean arsenic ~1,390 ng / g in liver biopsies of patients seen in West Bengal
J. Ind. Med. Assn. 96:4

GD18
Atherogenesis Indices Measured

- Lesion formation in aortic arch and valves
  - Fatty streak lesions as early indicator
- Plasma lipids
  - Cholesterol, phospholipids, triglycerides
- Vasoreactivity
  - Measure of endothelial dysfunction
Lesion Formation in Aortic Arches and Valves

Accelerated atherosclerosis (10 wk)

Exacerbated atherosclerosis (16 wk)

Reproductive Toxicology, 2007, 23(3):449-56
Plasma Lipid Analyses

10 wk

16 wk

Reproductive Toxicology, 2007, 23(3):449-56
Conclusions

• Transplacental arsenic exposure induces an early onset of atherosclerosis in ApoE-knockout mice without a hyperlipidemic diet and the intensity of disease increases with age

• Results support the hypothesis that prenatal arsenic exposure may be atherogenic in humans.
Atherogenesis 2 Hit Hypothesis

Atherosclerosis is the consequence of hyperlipidemia and chronic inflammation.
Liver Responses to Arsenic Exposure

Chronic arsenic exposure associated with hepatitis, cirrhosis, hepatocellular carcinoma

Prenatal arsenic exposure causes DNA methylation changes
Hypothesis

Prenatal arsenic exposure causes liver reprogramming that predisposes to altered inflammatory responses that contribute to atherogenesis.

Test using systems approach
MicroRNAs Regulate Gene Expression & Control Development

**Experimental Design 1**

1. **Treat pregnant dams**
   - Control
   - Arsenic
   - PND1
   - PND70

2. **Dissect livers - 3 mice / group**
   - PND1
   - PND70

3. **Purify total RNA from each liver**

4. **Microarray**
   - 44K NIA
   - 1.5K Exiqon

5. **2-color vs “mouse standard RNAs”**
Experimental Design 2

Microarray for mRNAs 44K NIA

2-color vs “mouse standard RNAs”

Microarray for microRNAs 1.5K Exiqon

ANOVA p<0.01

Changed mRNAs

Principal Component Analysis

Cluster Analysis

DAVID for Pathways

Venn Analysis for Overlap

Pathway Architect computational network

MicroRNA Classifier (BDSM) query

Sanger DB for mRNA targets
Venn Analysis Yields 51 Common Changed mRNAs

- Microarray for mRNAs 44K NIA
- Microarray for microRNAs 1.5K Exiqon

ANOVA p<0.01

2-color vs “mouse standard RNAs”

Venn Analysis for Overlap

PND1

PND70

763

51

848
Hierarchical Clustering
51 Common Changed mRNAs
Principal Component Analysis of “51 Common Genes”

- Microarray for mRNAs
  - 44K NIA
- 2-color vs “mouse standard RNAs”
- Microarray for microRNAs
  - 1.5K Exiqon
- ANOVA p<0.01
- Changed mRNAs
  - PND1
  - PND70
- Venn Analysis for Overlap
- Principal Component Analysis
Arsenic Alters Developmental Trajectory

![Venn Diagram and 3D Graph showing the impact of arsenic on developmental trajectory]
“51 gene” Interactome

Pathway Architect computational network,

HSPA8
IgM
HNF4A
LEF1
GSTA4
CDCA5
CYP7A1
UGDH
### Gene Nodes from “51”

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>PND1 As</th>
<th>PND70 As</th>
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<tbody>
<tr>
<td>HSPA8</td>
<td>Cognate HSP70</td>
<td>Up</td>
<td>Up</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
<td>Down</td>
<td>Down</td>
</tr>
<tr>
<td>HNF4A</td>
<td>controls the expression of several genes including:</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>LEF1</td>
<td>lymphoid enhancer-binding factor 1</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>GSTA4</td>
<td>glutathione S-transferase A4</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>CDCA5</td>
<td>regulator of sister chromatid cohesion</td>
<td>Down</td>
<td>Up</td>
</tr>
<tr>
<td>CYP7A1</td>
<td>rate limiting step and the major site of regulation of bile acid synthesis</td>
<td>Down</td>
<td>Up</td>
</tr>
<tr>
<td>UGDH</td>
<td>biosynthesis of glycosaminoglycans</td>
<td>Down</td>
<td>Up</td>
</tr>
</tbody>
</table>
Changed miRNAs Cluster Analysis

- Microarray for mRNAs 44K NIA
- 2-color vs “mouse standard RNAs”
- Microarray for microRNAs 1.5K Exiqon
- ANOVA p<0.01
- Changed microRNAs
- PND1
- PND70
- Cluster Analysis
Hierarchical Clustering

Arsenic-induced microRNA Changes

PND1:
11 annotated of 42 changed

PND70:
20 annotated of 106 changed

3 miRNAs up

8 miRNAs down

15 miRNAs down

5 miRNAs up
Principal Component Analysis
All changed mRNAs & miRNAs

Microarray for mRNAs
44K NIA

2-color vs “mouse standard RNAs”

Microarray for microRNAs
1.5K Exiqon

ANOVA p<0.01

Changed mRNAs

Principal Component Analysis

Changed microRNAs
Arsenic Exposure Alters Developmental Trajectories of mRNA and microRNA Expression

Principal Component Analysis

mRNA

microRNA
Genes Common to Changed mRNAs and Targets of miRNAs

Microarray for mRNAs 44K NIA

Microarray for microRNAs 1.5K Exiqon

2-color vs “mouse standard RNAs”

ANOVA p<0.01

Changed mRNAs

Changed microRNAs

Venn Analysis for Overlap

MicroRNA Classifier (BDSM) query Sanger DB for mRNA targets

DAVID for Pathways
DAVID Analysis of Intersection of mRNAs & microRNA Targets

Down by Arsenic PND1 (p < 0.05)

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<th>Category</th>
<th>Term</th>
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<th>Pvalue</th>
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<tbody>
<tr>
<td>KEGG_PATHWAY</td>
<td>MMU00010:GLYCOLYSIS / GLUCONEOGENESIS</td>
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Up by Arsenic PND70 (p < 0.05)

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<td>BIOCARTA</td>
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Interactome Analyses

- Microarray for mRNAs 44K NIA
- Microarray for microRNAs 1.5K Exiqon

2-color vs “mouse standard RNAs”

ANOVA $p<0.01$

- Changed mRNAs
- Pathway Architect computational network

PND1

PND70
PND1 Gene Interactome

IL1A
CD4
CD2
IL1B
IL2
TGFB1
IL1A
IL2RA
PPARG
HNF4A
SLC2A4
TNF
IFNG
IL2
IL1B
IL5
PND70 Gene Interactome

TNF

HSPA1B

HSPA8
Transcription Factor Analysis

PND1:
760 unique Entrez Ids
18 enriched TFs p<0.05

PND70:
690 unique Entrez Ids
31 enriched TFs p<0.05

developmental regulatory factors: Hox3A,
stress response factors: HSF, NFKB, AP1 ,
environmental agent response factors: AHR, HIF1
Cell cycle regulatory factors: E2F-1,
lipid metabolism gene regulator: SREBP-1
Plasma Markers of Liver Injury

Age 10 weeks

Relative level

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Arsenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td></td>
<td>!</td>
</tr>
<tr>
<td>AST</td>
<td></td>
<td>!</td>
</tr>
</tbody>
</table>

* Significant difference
Hsp70 & SREBP-1 Expression

Age 10 weeks
Post-natal Hsp70 Expression
Circulating Hsp70: A Potential Biomarker?

• Induces a pro-inflammatory state

• Activates macrophages
  – (Vega et al, J Immunol 2008 180:4299)

• Cytokine for both innate and adaptive immunity
  – (Chen & Cao, Eur J Immunol 2010 40:1541)

• Associated with increased risk of acute coronary syndrome
  – (Zhang et al, Cell Stress Chaperones 2010 15:675 )
Conclusions

- Prenatal arsenic exposure dysregulates liver development
- State of stress during early post-natal life
  - Elevated circulating Hsp70 may be a biomarker in early post-natal life
- Liver primed for inflammation as animals mature
- Additional insults predicted to cause continued liver injury and to provide inflammatory stimulus to initiate atherosclerotic process
- Disease outcome dependent on interaction of genetics and post-natal environment
Acknowledgements

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